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L4 15 DUP REM L3 (8 DUPLICATES REMOVED)

=> d l4 ibib ab 1-15

L4 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:522010 CAPLUS

DOCUMENT NUMBER: 137:104771

TITLE: Transgenic yeast expressing phosphatases for increase the efficiency of producing prenyl alcohol

INVENTOR(S): Tokuhiko, Kenro; Muramoto, Nobuhiko; Yamada, Yukio; Asami, Osamu; Hirai, Masana; Ohto, Chikara; Obata, Shusei; Muramatsu, Masayoshi

PATENT ASSIGNEE(S): Kabushiki Kaisha Toyota Chuo Kenkyusho, Japan; Toyota Jidosha Kabushiki Kaisha

SOURCE: PCT Int. Appl., 93 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002053751	A1	20020711	WO 2001-JP11223	20011220

W: CA, CN, IN, JP, US

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR

PRIORITY APPLN. INFO.: JP 2000-401515 A 20001228

JP 2000-401806 A 20001228

AB This invention provides a process of increasing prenyl alc.

**prodn.** by transformation of phosphatases into yeast. The DNA and protein sequences of 6 phosphatase from different sources were disclosed. The expression of phosphate resulted in the activation of geranylgeranyl pyrophosphatase activity which assocd. with resulted the increase of the prodn. of prenyl alc.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L4 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:522004 CAPLUS

DOCUMENT NUMBER: 137:89441

TITLE: Repression of expression of squalene synthase in Saccharomyces cerevisiae to increase the efficiency of

production of prenyl alcohol

INVENTOR(S): Ohto, Chikara; Obata, Shusei

PATENT ASSIGNEE(S): Toyota Jidosha Kabushiki Kaisha, Japan

SOURCE: PCT Int. Appl., 266 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002053747		20020711	WO 2001-JP11223	20011220
W: CA, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
JP 2002199883	A2	20020716	JP 2000-401701	20001228
PRIORITY APPLN. INFO.:			JP 2000-401701	A 20001228
			JP 2000-403067	A 20001228
			JP 2001-282978	A 20010918

AB This invention provides a process of repression of squalene synthase in *Saccharomyces cerevisiae* to increase the efficiency of prodn. of prenyl alc. The repression of squalene synthase expression was complemented by replacing the promoter of squalene synthase gene into GAL1 promoter. The isopentenyl diphosphate synthesis pathway assocd. enzymes, farnesyl diphosphate synthase, acetyl-CoA-acetyltransferase, hydroxymethylglutaryl CoA synthase, hydroxymethylglutaryl CoA reductase, mevalonate kinase, mevalonate phosphate kinase, isopentenyl diphosphate .DELTA.-isomerase from *Saccharomyces cerevisiae* were transformed into expression host. DNA sequences for farnesyl diphosphate synthase, geranylgeranyl diphosphate synthase and hydroxymethylglutaryl CoA reductase as well as the sequence of its mutated genes were provided. The invention also provides detailed description of expression vector construction for the enzymes expression in yeast.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L4 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2002 ACS  
 ACCESSION NUMBER: 2002:522002 CAPLUS  
 DOCUMENT NUMBER: 137:90182  
 TITLE: DNA and protein sequence of farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase and their uses for producing prenyl alcohol  
 INVENTOR(S): Ohto, Chikara; Obata, Shusei; Muramatsu, Masayoshi; Nishi, Kiyohiko; Totsuka, Kazuhiko  
 PATENT ASSIGNEE(S): Toyota Jidosha Kabushiki Kaisha, Japan  
 SOURCE: PCT Int. Appl., 337 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: Japanese  
 FAMILY ACC. NUM. COUNT: 3  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002053746	A1	20020711	WO 2001-JP11214	20011220
W: CA, CN, IN, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
PRIORITY APPLN. INFO.:			JP 2000-403067	A 20001228
AB This invention provides DNA and protein sequence farnesyl diphosphate synthase of <i>Saccharomyces cerevisiae</i> and geranylgeranyl diphosphate synthase of <i>E. coli</i> . The invention also provides the process of cloning of farnesyl diphosphate synthase, acetyl-CoA-acetyltransferase, hydroxymethylglutaryl CoA synthase, hydroxymethylglutaryl CoA reductase, mevalonate kinase, mevalonate phosphate kinase, isopentenyl diphosphate .DELTA.-isomerase from <i>Saccharomyces cerevisiae</i> . The invention also provides detailed description of expression vector construction for the enzymes expression in yeast and <i>E. coli</i> . The enzymes can be used for biosynthesis of prenyl alc. such as farnesol and nerolidol.				
REFERENCE COUNT: 7			THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE	

FORMAT

L4 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:276135 CAPLUS  
 DOCUMENT NUMBER: 136:291636  
 TITLE: Improved **ethanol production** using thermophilic strains of **Bacillus**  
 INVENTOR(S): Javed, Muhammad; Cusdin, Fiona; Milner, Paul; Green, Edward  
 PATENT ASSIGNEE(S): Elsworth Biotechnology Limited, UK  
 SOURCE: PCT Int. Appl., 44 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002029030	A2	20020411	WO 2001-GB4434	20011005
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002081677	A1	20020627	US 2001-971361	20011005
PRIORITY APPLN. INFO.:			GB 2000-24554	A 20001006
			US 2000-247017P	P 20001113

AB The present invention relates to the prodn. of ethanol as a product of fermn of a thermol. **Bacillus** sp. In particular this invention relates to a novel method of gene inactivation and gene expression based upon homologous recombination. The invention shows that **ethanol prodn.** may be improved through stabilization of a ldh (lactate dehydrogenase) gene mutation using transposon mutagenesis and homologous recombination in **Bacillus** strain TN. Furthermore, the PDC operon contg. pdc (pyruvate **decarboxylase**) gene from Zymomonas mobilis and adh (alc. dehydrogenase) gene from **Bacillus** strain LN may be expressed in the said strain for improved **ethanol prodn.**  
 . The invention further claims the prodn. of ethanol using fermn. at a temp. between 40-75oC and a pH of 5.5-7.5. with air sparging in the culture such that the redox potential is between -360 and -400 mV. Furthermore, a process for continuous prodn. of ethanol in which the feed dilyn. rates are between 0.3-0.8 h<sup>-1</sup> is provided. The inventors have produced sporulation deficient variants of a thermophilic, facultatively anaerobic, Gram-pos. bacterium which exhibit improved **ethanol prodn.**-related characteristics.

L4 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1  
 ACCESSION NUMBER: 2002:209986 CAPLUS  
 DOCUMENT NUMBER: 136:368511  
 TITLE: Flux through citrate synthase limits the growth of ethanologenic Escherichia coli KO11 during xylose fermentation  
 AUTHOR(S): Underwood, S. A.; Buszko, M. L.; Shanmugam, K. T.; Ingram, L. O.  
 CORPORATE SOURCE: Institute of Food and Agricultural Sciences, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, 32611, USA  
 SOURCE: Applied and Environmental Microbiology (2002), 68(3), 1071-1081  
 CODEN: AEMIDF; ISSN: 0099-2240  
 PUBLISHER: American Society for Microbiology  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB Previous studies have shown that high levels of complex nutrients (Luria

broth or 5% corn steep liquor) were necessary for rapid **ethanol prodn.** by the ethanologenic strain *Escherichia coli* KO11. Although this strain is prototrophic, cell d. and **ethanol prodn.** remained low in mineral salts media (10% xylose) unless complex nutrients were added. The basis for this nutrient requirement

was

identified as a regulatory problem created by metabolic engineering of an ethanol pathway. Cells must partition pyruvate between competing needs for biosynthesis and regeneration of NAD+. Expression of low-Km

*Zymomonas*

*mobilis* **pdc** (pyruvate **decarboxylase**) in KO11 reduced the flow of pyruvate carbon into native fermn. pathways as desired, but it also restricted the flow of carbon skeletons into the 2-ketoglutarate arm of the tricarboxylic acid pathway (biosynthesis). In mineral salts medium contg. 1% corn steep liquor and 10% xylose, the detrimental effect of metabolic engineering was substantially reduced by addn. of pyruvate. A similar benefit was also obsd. when acetaldehyde, 2-ketoglutarate, or glutamate was added. In *E. coli*, citrate synthase links the cellular abundance of NADH to the supply of 2-ketoglutarate for glutamate biosynthesis. This enzyme is allosterically regulated and inhibited by high NADH concns. In addn., citrate synthase catalyzes the first committed step in 2-ketoglutarate synthesis. Oxidn. of NADH by added acetaldehyde (or pyruvate) would be expected to increase the activity of *E. coli* citrate synthase and direct more carbon into 2-ketoglutarate, and this may explain the stimulation of growth. This hypothesis was tested, in part, by cloning the *Bacillus subtilis* **citZ** gene encoding an NADH-insensitive citrate synthase. Expression of recombinant **citZ** in

KO11

was accompanied by increases in cell growth and **ethanol prodn.**, which substantially reduced the need for complex nutrients.

REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L4 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:507865 CAPLUS

DOCUMENT NUMBER: 135:104937

TITLE: **Ethanol production** by thermophilic strains of *Bacillus* sp.

INVENTOR(S): Green, Edward; Baghaei-Yazdi, Namdar; Javed, Muhammad

PATENT ASSIGNEE(S): Elsworth Biotechnology Limited, UK

SOURCE: PCT Int. Appl., 30 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001049865	A1	20010712	WO 2001-GB36	20010105
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
US 2002034816	A1	20020321	US 2001-754083	20010105
PRIORITY APPLN. INFO.:			GB 2000-185	A 20000106
			US 2000-177199P	P 20000121
AB	This invention relates to <b>ethanol prodn.</b> as a product			

of bacterial fermm. In particular, the invention relates to **ethanol prodn.** by thermophilic strains of **Bacillus** sp. The invention describes the incorporation of heterologous gene *pdcs* of *S. cerevisiae* or *Z. mobilis* into the chromosome of the gram-pos. bacterium. The bacterium is transformed with plasmid *pFC1*, more preferably with *pFC1-PDC1*. The invention further claims the prodn. of ethanol at a temp. between 40-750C.

L4 ANSWER 7 OF 15 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1998-04018 BIOTECHDS

TITLE: Metabolic engineering of bacteria for **ethanol production**;

by transformation with the *Zymomonas mobilis* pyruvate-decarboxylase gene ; a review

AUTHOR: Ingram L O; Gomez P F; Lai X; Moniruzzaman M; Wood B E; Yomano L P; York S W

CORPORATE SOURCE: Univ.Florida-Inst.Food-Agr.Sci.

LOCATION: Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, USA.

Email: [lingram@micro.ifas.ufl.edu](mailto:lingram@micro.ifas.ufl.edu)

SOURCE: Biotechnol.Bioeng.; (1998) 58, 2-3, 204-14

CODEN: BIBIAU

ISSN: 0006-3592

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The metabolic engineering of bacteria to convert lignocellulose into ethanol is reviewed. Topics include: lignocellulose is a challenging substrate for bioconversion; dilute hydrolysis of hemicellulose; enzymatic hydrolysis of cellulose; nutrients for lignocellulose-based fermentation; a hybrid approach for lignocellulose conversion to ethanol;

genetic engineering of bacteria to ferment hemicellulose sugars; improvements in ethanologenic *Escherichia coli*; fermentation of hemicellulose-derived sugars; genetic engineering of bacteria for cellulose fermentation; process optimization for cellulose fermentation;

**ethanol production** acid-treated bagasse;

**ethanol production** from office mixed waste-paper; other

improvements in the biomass conversion; fermentation of di-, tri-, and tetrasaccharides; and nutrients for the fermentation of lignocellulosic sugars. For **ethanol production**, the *Zymomonas*

*mobilis* pyruvate-decarboxylase (EC-4.1.1.1) gene has been expressed in *E. coli*, *Erwinia chrysanthemi*, *Klebsiella planticola*, *Klebsiella oxytoca*, *Enterobacter cloacae* and **Bacillus subtilis**.

(62 ref)

L4 ANSWER 8 OF 15 CEABA-VTB COPYRIGHT 2002 DECHEMA

ACCESSION NUMBER: 1997(06):4068 CEABA-VTB FILE SEGMENT B

DOCUMENT NUMBER: CEABA: 1997:1421890

TITLE: **Ethanol production** in gram-positive microbes

AUTHOR: Ingram, L. O N; Barbosa-Alleyne, M. D. F. (Univ. Florida, Gainesville, FL, USA)

SOURCE: US Patent (1996) US 5482846 (Appl. US 220072 Filed 30 Mar 1994)

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

AB A gram-positive bacterium which was selected from **Bacillus subtilis** or **Bacillus polymyxa** is disclosed which was transformed with *Zymomonas mobilis* genes encoding alcohol dehydrogenase and pyruvate **decarboxylase**. Expression of the genes within the transformant allows the bacterium to produce ethanol as a fermentation product.

L4 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2002 ACS

DUPLICATE 2

ACCESSION NUMBER: 1996:103844 CAPLUS  
 DOCUMENT NUMBER: 124:143770  
 TITLE: **Ethanol production in**  
 Gram-positive microbes  
 INVENTOR(S): Ingram, Lonnie O'Neal; Barbosa-Alleyne, Maria D. F.  
 PATENT ASSIGNEE(S): University of Florida, USA  
 SOURCE: U.S., 11 pp. Cont.-in-part of U.S. Ser. No. 26,051.  
 CODEN: USXXAM  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 10  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5482846	A	19960109	US 1994-220072	19940330
US 5000000	A	19910319	US 1989-352062	19890515
US 5424202	A	19950613	US 1992-846344	19920306
CN 1070424	A	19930331	CN 1992-101877	19920318
CN 1065915	B	20010516		
US 5487989	A	19960130	US 1992-946290	19920917
WO 9527064	A1	19951012	WO 1995-US4012	19950330
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, UZ, VN RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9522034	A1	19951023	AU 1995-22034	19950330
US 5916787	A	19990629	US 1995-475925	19950607
AU 9918586	A1	19990909	AU 1999-18586	19990305
CN 1342773	A	20020403	CN 2000-131779	20001020
PRIORITY APPLN. INFO.:				
			US 1988-239099	B2 19880831
			US 1989-352062	A2 19890515
			US 1990-624227	B2 19901207
			US 1991-670821	B2 19910318
			US 1992-846344	A2 19920306
			US 1992-946290	A2 19920917
			US 1993-260517	A2 19930305
			US 1990-624277	B2 19901207
			US 1993-26051	A2 19930305
			US 1994-220072	A 19940330
			WO 1995-US4012	W 19950330
			AU 1996-61946	A3 19960808

AB The subject invention concerns the transformation of Gram-pos. bacteria with heterologous genes which confer upon these microbes the ability to produce EtOH as a fermn. product. Specifically exemplified is the transformation of bacteria with genes, obtainable from Zymomonas mobilis, which encode pyruvate **decarboxylase** and alc. dehydrogenase.

L4 ANSWER 10 OF 15 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1996-13636 BIOTECHDS

TITLE: Production of recombinant bacterial cellulases by  
 ethanologenic bacteria: evaluation for cellulose  
 fermentation

;  
 cellulase expression in Escherichia coli for improved  
**ethanol production** (conference abstract)

AUTHOR: Wood B E; Ingram L O

CORPORATE SOURCE: Univ.Florida

LOCATION: University of Florida, Gainesville, FL 32611, USA.

SOURCE: Abstr.Gen.Meet.Am.Soc.Microbiol.; (1996) 96 Meet., 566

CODEN: 0005P

ISSN: 0067-2777

American Society for Microbiology, 96th General Meeting, New Orleans, LA, 19-23 May, 1996.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Previously, *Escherichia coli* KO11 was engineered for fermentation of mixtures of pentose and hexose sugars, and *Klebsiella oxytoca* P2 was engineered for fermentation of cellobiose (from cellulose) to ethanol by integrating the *Zymomonas mobilis* genes for pyruvate-decarboxylase (pdc, EC-4.1.1.1) and alcohol-dehydrogenase (adh, EC-1.1.1.1). In this study, production of recombinant cellulase (EC-3.2.1.4) in KO11 was evaluated during pentose fermentation as a source of supplemental enzymes for cellulose fermentations. Cellulase genes from *Cellulomonas fimi*, *Clostridium thermocellum*, *Erwinia* sp. and *Bacillus subtilis* were tested. In some cases, high levels of cellulase were produced without compromising the ability of KO11 to produce ethanol. Results indicated that it was possible to make significant reductions in the requirement for fungal enzymes by this approach, and show the potential for manufacturing recombinant protein products with ethanol. (0 ref)

L4 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2002 ACS

DUPLICATE 3

ACCESSION NUMBER: 1995:992753 CAPLUS

DOCUMENT NUMBER: 124:28129

TITLE: Ethanol production with recombinant Gram-positive microbes expressing exogenous pyruvate decarboxylase and alcohol dehydrogenase genes

INVENTOR(S): Ingram, Lonnie O'Neal; Barbosa-Alleyne, Maria de F. S.

PATENT ASSIGNEE(S): University of Florida, USA

SOURCE: PCT Int. Appl., 33 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 10

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9527064	A1	19951012	WO 1995-US4012	19950330
W:	AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, UZ, VN			
RW:	KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
US 5482846	A	19960109	US 1994-220072	19940330
AU 9522034	A1	19951023	AU 1995-22034	19950330
PRIORITY APPLN. INFO.:			US 1994-220072	A 19940330
			US 1988-239099	B2 19880831
			US 1989-352062	A2 19890515
			US 1990-624227	B2 19901207
			US 1991-670821	B2 19910318
			US 1992-846344	A2 19920306
			US 1992-946290	A2 19920917
			US 1993-260517	A2 19930305
			WO 1995-US4012	W 19950330

AB The subject invention concerns the transformation of Gram-pos. bacteria with heterologous genes which confer upon these microbes the ability to produce ethanol as a ferment. product. Specifically exemplified is the transformation of bacteria with genes, obtainable from *Zymomonas mobilis*, which encode pyruvate decarboxylase and alc. dehydrogenase. A recombinant *Bacillus subtilis* expressing *Z. mobilis* pdc and adhB genes was created.

L4 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2002 ACS

DUPLICATE 4

ACCESSION NUMBER: 1995:5540 CAPLUS

DOCUMENT NUMBER: 122:24802

TITLE: Expression of the *Zymomonas mobilis* alcohol dehydrogenase II (adhB) and pyruvate decarboxylase (pdc) genes in *Bacillus*

AUTHOR(S): Barbosa, Maria de F. S.; Ingram, L. O.

CORPORATE SOURCE: Dep. Microbiol. Cell Sci., Univ. Florida, Gainesville, FL, USA

SOURCE: Current Microbiology (1994), 28(5), 279-82  
CODEN: CUMIDD; ISSN: 0343-8651

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The genes encoding *Zymomonas mobilis* pyruvate decarboxylase (pdc) and alc. dehydrogenase II (adhB) were expressed in *Bacillus subtilis* YB886 (pLOI500) under the control of a *Bacillus* SPO2 phage promoter and caused a 50% redn. of growth rate compared with the unmodified vector. Expression was further confirmed by Western blots, activity stains of native gels, and in vitro measurements of alc. dehydrogenase activity. Addnl. strains of *Bacillus* were also transformed, and all produced similar but low levels of these enzymes. Although higher specific activities will be required for efficient ethanol prodn., no fundamental barriers exist to the expression of these *Z. mobilis* genes in *Bacillus*. Two abundant new proteins (ca. mass 33,000 daltons and 14,000 daltons) were obsd. in Coomassie Blue-stained gels; they are similar in size to the proteins induced by recombinant products in *Escherichia coli*.

L4 ANSWER 13 OF 15 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 94:535177 SCISEARCH

THE GENUINE ARTICLE: PD286

TITLE: CONSTRUCTION OF RECOMBINANT PLASMIDS FOR EFFICIENT EXPRESSION OF THE PYRUVATE DECARBOXYLASE GENE (PDK) FROM *ZYMONONAS-MOBILIS* IN *BACILLUS* -*SUBTILIS*

AUTHOR: DANILEVICH V N (Reprint); DUZHII D E; BRAGA E A

CORPORATE SOURCE: MOSCOW GENET & SELECT IND MICROORGANISMS INST, MOSCOW 113545, RUSSIA (Reprint)

COUNTRY OF AUTHOR: RUSSIA

SOURCE: MOLECULAR BIOLOGY, (JAN/FEB 1994) Vol. 28, No. 1, Part 2, pp. 105-110.  
ISSN: 0026-8933.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: ENGLISH

REFERENCE COUNT: 28

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The pdk gene from *Zymomonas mobilis* localized in a 4.7-kbp SphI fragment of plasmid pB201 was subcloned into the SmaI site of the M13mp19 vector using the DraI restriction endonuclease. The M13mp19 derivatives obtained, carrying a 1.8-kbp DraI fragment in opposite orientations, were used to sequence the pdk gene beginning and end (about 250 bp each) and for site-directed mutagenesis. Using polymerase chain reaction with synthetic oligonucleotide primers, a BamHI site was created in front of the pdk gene initiating codon. The BamHI fragment harboring the pdk gene was cloned into shuttle vector pCB20 under the control of "expression unit" EU19035 containing bacillar vegetative promoter and ribosome-binding site (RBS). The pdk gene expression was studied in the recombinant plasmid pCB20pdkI, a derivative of pCB20, which was shown to yield a high level of pyruvate decarboxylase [EC 4.1.1.1] synthesis in *Bacillus subtilis*. However, this plasmid strongly inhibited the *Escherichia coli* cell growth and was eliminated from the cells at a high frequency.

L4 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1992:649986 CAPLUS

DOCUMENT NUMBER: 117:249986

TITLE: Ethanol production by bacteria

carrying foreign genes for alcohol dehydrogenase and pyruvate **decarboxylase**

INVENTOR(S): Ingram, Lonnie O.; Beall, David S.; Burchhardt, Gerhard F. H.; Guimaraes, Walter V.; Ohta, Kazuyoshi; Wood, Brent E.; Shanmugam, Keelnatham T.; Fowler, David A.; Ben-Bassat, Arie

PATENT ASSIGNEE(S): University of Florida, USA; Bioenergy International, L.C.

SOURCE: PCT Int. Appl., 153 pp.  
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 10

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9216615	A1	19921001	WO 1992-US1807	19920318
W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US				
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
US 5424202	A	19950613	US 1992-846344	19920306
AU 9217794	A1	19921021	AU 1992-17794	19920318
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CN 1070424	A	19930331	CN 1992-101877	19920318
CN 1065915	B	20010516		
EP 576621	A1	19940105	EP 1992-910933	19920318
EP 576621	B1	20010228		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
BR 9205782	A	19940726	BR 1992-5782	19920318
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NO 9303178	A	19931108	NO 1993-3178	19930907
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US 1989-352062	A2	19890515
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AB Bacterial hosts, excluding Escherichia coli, expressing heterologous genes

for alc. dehydrogenase (I) and pyruvate **decarboxylase** (II) are used for manuf. of EtOH. II is used to prevent accumulation of acid metabolites. Plasmids, e.g. pLOI555 carrying genes for I and II of Zymomonas mobilis driven by the lac promoter, are provided for prepn. of the host. The method is further improved by transforming the host with genes for proteins that facilitate transport and metab. of oligosaccharides, e.g., of C5-6 sugars, which host is, preferably, also expressing a heterologous gene for a polysaccharase such as a cellulolytic

enzyme, a xylanolytic enzyme, or a starch-degrading enzyme. These hosts also preferably express heterologous genes for polysaccharide- degrading enzymes (e.g. those degrading cellulose, xylans, or starch). A cost-effective fermn. process for manufg. EtOH from oligosaccharide feedstocks using a single, genetically engineered microorganism is also disclosed. An ethanologenic strain Klebsiella oxytoca M5A1(pLOI555) was prepd. and was further transformed with plasmid pLOI2003 encoding xylanase

(gene xynZ) and xylosidase (gene xylB) of Clostridium thermocellum to obtain a transformant capable of converting xylan to EtOH.

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THE GENUINE ARTICLE: FK459

TITLE: EXPRESSION OF AN L-ALANINE DEHYDROGENASE GENE IN

ZYMOMONAS-MOBILIS AND EXCRETION OF L-ALANINE  
 AUTHOR: ULLMANNBUSCH I; SAHM H; SPRENGER G A (eprint)  
 CORPORATE SOURCE: FORSCHUNGSZENTRUM JULICH GMBH, INST BIOTECHNOL, POSTFACH  
 1913, W-5170 JULICH, GERMANY  
 COUNTRY OF AUTHOR: GERMANY  
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 No. 5, pp. 1360-1366.  
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\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB An approach to broaden the product range of the ethanologenic,  
 gram-negative bacterium *Zymomonas mobilis* by means of genetic engineering  
 is presented. Gene *alaD* for L-alanine dehydrogenase (EC 1.4.1.1) from  
*Bacillus sphaericus* was cloned and introduced into *Z. mobilis*.  
 Under the control of the strong promoter of the pyruvate  
**decarboxylase** (*pdc*) gene, the enzyme was expressed up to a  
 specific activity of nearly 1- $\mu$ -mol . min<sup>-1</sup> . mg of protein<sup>-1</sup> in  
 recombinant cells. As a result of this high L-alanine dehydrogenase  
 activity, growing cells excreted up to 10 mmol of alanine per 280 mmol of  
 glucose utilized into a mineral salts medium. By the addition of 85 mM  
 NH<sub>4</sub><sup>+</sup> to the medium, growth of the recombinant cells stopped, and up to 41  
 mmol of alanine was secreted. As alanine dehydrogenase competed with  
 pyruvate **decarboxylase** (PDC) (EC 4.1.1.1) for the same substrate  
 (pyruvate), PDC activity was reduced by starvation for the essential PDC  
 cofactor thiamine PP(i). A thiamine auxotrophy mutant of *Z. mobilis*  
 which  
 carried the *alaD* gene was starved for 40 h in glucose-supplemented  
 mineral  
 salts medium and then shifted to mineral salts medium with 85 mM NH<sub>4</sub><sup>+</sup> and  
 280 mmol of glucose. The recombinants excreted up to 84 mmol of alanine  
 (7.5 g/liter) over 25 h. Alanine excretion proceeded at an initial  
 velocity of 238 nmol . min<sup>-1</sup> . mg [dry weight]<sup>-1</sup>. Despite this high  
 activity, the excretion rate seemed to be a limiting factor, as the  
 intracellular concentration of alanine was as high as 260 mM at the  
 beginning of the excretion phase and decreased to 80 to 90 mM over 24 h.